USE OF THE RAPID WHOLE-BLOOD TEST

For Pullorum Disease

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USE OF THE RAPID WHOLE-BLOOD TEST FOR PULLORUM DISEASE

By Hubert Bunyea, *veterinarian, Pathological Division, Bureau of Animal Industry*

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NEED FOR CONTROL OF PULLORUM DISEASE

The general prevalence of pullorum disease, the heavy losses that it causes, and the insidious means of its dissemination combine to make its control of vital importance to a profitable poultry industry. The infection is harbored and perpetuated in the ovaries of infected hens and transmitted by them to their offspring through the egg. The control of the disease involves the detection of infected hens and their elimination from the breeding flock (fig. 1). The objective of pullorum disease control, namely, the production of healthy, vigorous chicks, is essentially the same throughout the industry. The technique of pullorum disease control, however, varies considerably in different parts of the United States. An important influence is the distribution of poultry breeding stock in various regions.

The organized control of pullorum disease began with the introduction of the tube agglutination test in 1913, by means of which infected hens could be detected. Four years later the intradermic or so-called wattle test was devised for the detection of carrier hens; but this method failed to achieve wide acceptance, as its reliability was open to considerable question. In 1927 the rapid, serum test was announced as a step in advance of the then-prevailing methods for the diagnosis of pullorum disease carriers. This method possessed some features which rendered it virtually a laboratory test. Its usefulness was thus limited to certain working conditions. Realizing the difficulties involved in conducting a Nation-wide campaign against pullorum disease under such conditions, the Bureau of Animal Industry directed its efforts toward the development of a test method that could

be used on farms as well as in the laboratory. The stained-antigen, rapid, whole-blood agglutination test for pullorum disease resulted in 1931 from these efforts.

Where poultry flocks are reasonably well concentrated, where distances are moderate, or where the shipment of blood samples used in the test is facilitated by good transportation, it is feasible in some States to combat pullorum disease by means of testing operations conducted in a centralized laboratory. On the other hand, where flocks are scattered, as is the case in many of the larger States, and where they are less accessible to a central point of operation, the control of pullorum disease by means of laboratory tests presents serious problems incident to obtaining and identifying the blood samples and transmitting them to the laboratory in suitable time and condition to make satisfactory tests. It is under such conditions that the more

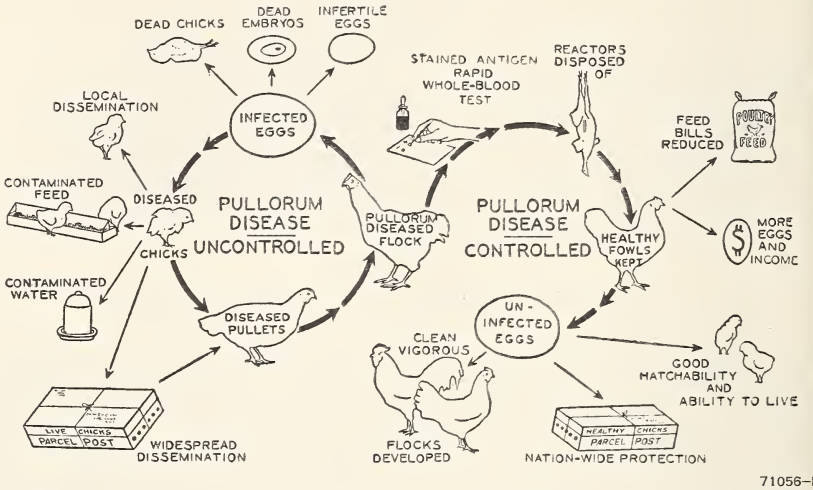

FIGURE 1.—Comparison of injurious effects of uncontrolled pullorum disease with benefits resulting from the rapid, whole-blood test and disposal of reactors.

recently developed stained-antigen, rapid, whole-blood method has been utilized very effectively.

With the inauguration, July 1, 1935, of the National Poultry Improvement Plan, the stained-antigen, rapid, whole-blood agglutination test was included as one of the optional methods of testing for pullorum disease control. This method is being used in many of the States where the national plan has been adopted. Within the U. S. Pullorum-Passed and U. S. Pullorum-Clean classes provided for by the plan, the testing of fowls for pullorum disease is done only by a livestock sanitary authority, an official of the State college of agriculture, or similarly authorized State employee. But in the U. S. Pullorum-Tested class, which constitutes approximately 70 percent of the pullorum disease control work conducted under the national plan, the limited personnel of the official State agency in many instances could not possibly cope with the task. The national plan provides for this circumstance by permitting the testing of fowls in this class to be done also—

by a pullorum-testing agent who shall be required to take a course of training in pullorum testing prescribed by the livestock sanitary authorities or officials of the State college of agriculture, and shall be required to pass an examination and be authorized by the official State agency to do pullorum-testing work. The livestock sanitary authorities, officials of the State college of agriculture, or similarly authorized State employee, under whose supervision the pullorum-testing work is carried on, shall assume responsibility for the accuracy of the testing work done by the pullorum-testing agents.

Of the 44 States cooperating in the work of the National Poultry Improvement Plan during the fiscal year ended June 30, 1939, 42 States had official pullorum disease testing programs in line with the provisions of the plan. A total of 1,008 persons were authorized by the official agencies of 22 States as pullorum-testing agents. This use of pullorum-testing agents has resulted in a more extensive pullorum-control program than would be possible if all the work were required to be done by the limited staff employed by the official State agency.

QUALIFICATIONS OF PULLORUM DISEASE TESTERS

Following are the qualities considered essential in a good pullorum-testing agent. Physically, he must be endowed with steady nerves to perform the mechanical movements of bleeding the bird and applying the test with minimum danger of error or accident or undue loss of time or blood, keen vision to detect the variable phenomenon of agglutination, and endurance to maintain an efficient performance of the test until disposal of the last case.

He should possess the attributes of patience, thoroughness, and discrimination to arrive at a reliable diagnosis, and carefully but unhesitatingly formulate his judgment.

A high degree of native intelligence is important. He should be at least 21 years old to bring to his work a measure of mature appreciation of its importance and exacting requirements. Integrity and reliability are indispensable assets in winning the confidence of others. Furthermore, he should have had experience in handling fowls, and he should have some knowledge of poultry husbandry, as it is desirable to take all possible precautions to prevent the blood-testing of a flock from interfering with normal egg production.

The qualification of a pullorum-testing agent that more than any other thing governs his fitness for the work is the training that he receives from the livestock sanitary authorities or the officials of the State college of agriculture, since this involves the obligation on his part to show his proficiency by passing an examination before he shall receive a permit to test poultry in his State. There is no place in the pullorum disease control program for a poorly trained or untrained technician. Such a person could leave behind him a wake of disaster, in the form of unrecognized pullorum disease carriers, that would tend to destroy the confidence of the poultryman in the entire control program.

The trained man is competent to exercise proper care and intelligent discrimination in making the test. It is difficult to separate training from experience, for the latter is in a very real sense a part of the former. Having been schooled in the fundamentals of the stained-antigen, rapid, whole-blood test, the technician proceeds to perfect himself in the work by testing and more testing. For this reason, it is suggested that the poultry-testing school courses conducted by the various State agencies should include a considerable amount of field

clinical work under proper auspices prior to the granting of a license or permit. The cooperating official State agencies in the National Poultry Improvement Plan are recognizing more and more the desirability of having a qualified person spend at least 1 day in the field with each pullorum-testing agent at the beginning of the testing season to assist him in developing an efficient technique, and to make sure that he fully understands the program and appreciates his responsibilities. His work during the testing season may well be kept under official observation, and the continuance or renewal of his testing permit conditioned on the proficiency shown.

EQUIPMENT AND PROCEDURE[1]

Since the stained-antigen, rapid, whole-blood agglutination test for pullorum disease was originally announced, this method has acquired much favor with the poultry industry and has been officially adopted for the control of pullorum disease under the National Poultry Improvement Plan in a considerable number of the participating States. The use of the test has resulted in the development of certain kinds of testing equipment, some of which are satisfactory, others not so efficient. Also certain procedures have grown up around the test, concerning which some comments should be made.

The growth of these different ideas and their adoption in various parts of the country have made it desirable to issue a detailed statement of the correct technique by which the test should be conducted, as well as to describe the most suitable testing equipment. It is also desirable to correct certain erroneous ideas and faulty practices which have crept in.

There is no wish to discourage the use of ingenious or original contrivances or unique procedures, so far as they are in conformity with the accepted technique of testing. However, observations have shown that the efficient application of the test lies along definite lines. It is the purpose of this publication to offer suggestions leading to the provision of suitable testing equipment and the application of correct methods, as well as the elimination of equipment and methods that have proved to be undesirable.

The general routine of preliminary steps usually involves the following procedures: Confining and catching the birds, placing them on a holding table or otherwise confining them while under test, bleeding them, and taking the blood sample. After these steps are taken the test is performed and disposition is made of the reactors.

CONFINING AND CATCHING THE BIRDS

Various methods are in use for this purpose. The flock is usually kept in the house on the day of the test. If the testing is done in the house, a roll of 5-foot poultry wire is uncoiled in an upright position in one corner, with one end stapled to the wall and the other end extending along the end wall to make a corral or enclosure for the birds. The fowls are then driven slowly into this corral, where they may be easily caught and handed over the wire for testing and leg-banding.

It is best not to test in the poultry house as there is invariably considerable dust in the litter, and dust interferes appreciably with the test. Furthermore, the illumination in the house is usually insufficient for reading the test.

[1] See list of equipment on p. 20.

If the test is to be done out of doors, a catching coop, or a series of coops, with removable ends is usually placed at the poultry-house entrance. Enough birds are driven out to fill the catching coops. A satisfactory coop, with an end door and a top door, can be constructed of wood strips, or wood and poultry wire. Portable and collapsible coops are extremely convenient for hatcherymen who test the flocks from which they obtain hatching eggs. Collapsible, sectional, wire, catching coops may be purchased. These may be folded for transportation, and they occupy a comparatively small space in the back of the truck.

It is sometimes the practice to catch the birds early in the morning and confine them in coops pending the arrival of the tester. This practice is to be discouraged, as it is hard on the birds to be crowded up for a long period without food or water. Furthermore, if the tester

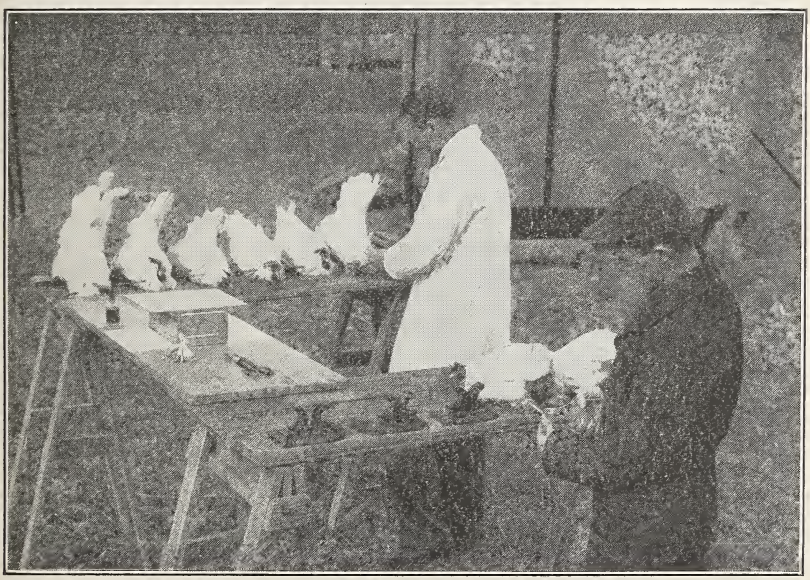

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FIGURE 2.—A practical type of portable holding table with testing table across the ends. Note pairs of screen-door bumpers for clamping legs of each chicken to the table, also the spiral spring over necks of confined chickens for added restraint. Plain glass testing plate with white background and warm-water can is in use. Banding is being done by an assistant at right.

does not come on the day appointed, the flock is subjected to unnecessary hardship if caught in advance. Much of the loss of egg production sometimes attributed to pullorum testing may be avoided if the birds are handled humanely.

HOLDING THE BIRDS

In order to expedite the testing of a flock, it is desirable to have suitable equipment for retaining the birds under test until the diagnosis is made. Various types of equipment have been used for this purpose, such as numbered trap nests, a series of numbered egg crates or orange boxes, metal cones, feed bags hung along the wall, and holding

tables (fig. 2). The last-named type of equipment is by far the most common and possesses the advantage of restraining the birds while they are being bled and banded. Much ingenuity has been devoted by various testers to the development of portable holding tables (fig. 2, also cover page). Some are built on ordinary folding ironing boards. The essential features of a good holding table are that it will effectively restrain a sufficient number of birds for practical working conditions and that it can be folded into a compact unit for transportation and can be easily kept in a sanitary condition. Various restraining contrivances are in use, the basic principle being to hold

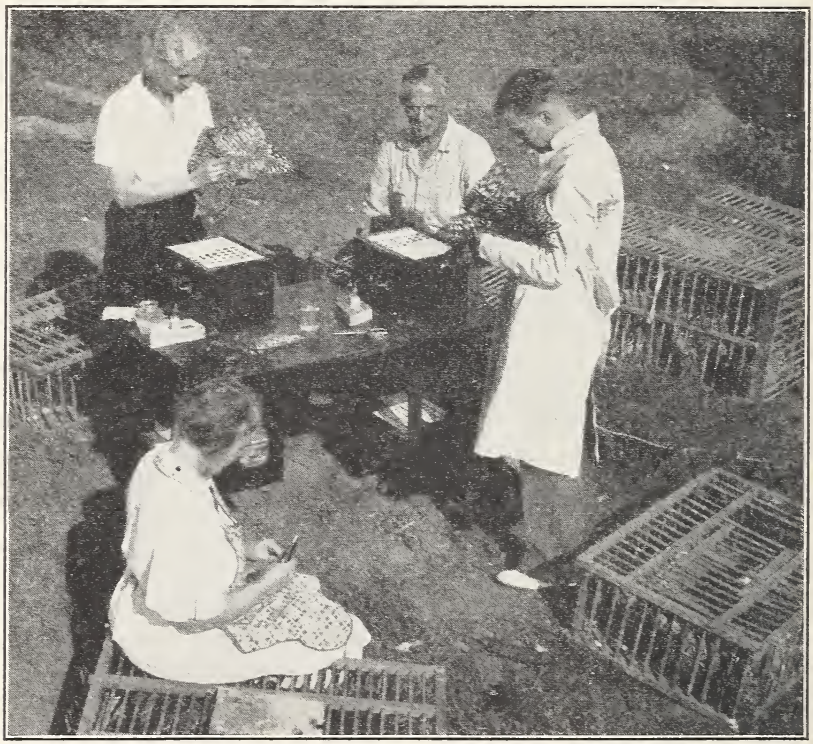

FIGURE 3.—Simple equipment for testing chickens for pullorum disease. Birds are held in hand while being tested. Note extra coops provided for restraining reactors. Banding is done by two assistants, one at center and the other in left foreground.

the bird's legs securely. Leather thongs, wooden turn buttons, and spring screen-door bumpers are in common use for clamping the bird's legs to the table. It is also advisable to keep the bird's wings from flapping. This is accomplished by stretching a broad band of inner-tube rubber or a spiral spring from one end of the table to the other. The birds' necks are placed under this rubber strip or spring and their feet secured. They are then well restrained for testing and banding.

If a holding table is not used the operator may hold the bird in his left hand and bleed it with the instrument in his right hand, as shown in figure 3. After the blood sample has been obtained, the bird may be placed in a designated box or trap nest pending its final disposition.

A fowl to be bled is caught in the left hand by both wings and held feet up, with the body balanced on the free fingers. The bird will not struggle in this position, and the wing to be bled may be spread open by the left thumb, and the drop of blood obtained for the test.

BLEEDING THE BIRDS

The blood sample is generally obtained from the brachial vein of the wing. The vein is punctured with a needle or other sharp instrument, near the point of the elbow, where the vein is close to the surface. The reason for selecting this site is that its superficial position enables the operator to puncture the vein without causing any appreciable subcutaneous hemorrhage. The vein should not be punctured in the fleshy portion of the member, as the subcutaneous hemorrhage (blood leaking out under the skin) would make the carcass objectionable for food purposes if sold immediately after the test. Also the operator should avoid puncturing through the under side of the blood vessel, as this may cause hemorrhages under the skin.

The practice exists in some localities of bleeding birds from the tip of the comb or the wattles. This obviates the objection of blemishing the wing but creates a wound and affords an entry for pox infection if present. However, for the purposes of the test the point of origin of the blood is not important so long as it is possible to utilize the blood loop in measuring the quantity taken up for testing, and does not occasion a serious wound.

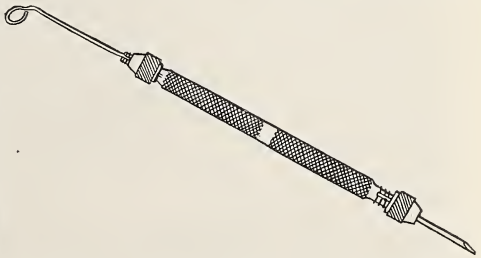

FIGURE 4.—A convenient type of bleeding instrument with metal chuck at each end. Stylet inserted in one end and blood loop in the other.

To avoid subcutaneous hemorrhage of the wing, a puncture should be made so as to spread the opening in the skin. This is best accomplished by using a stylet or other piercing instrument with a beveled point, similar to a large-gage, sharp, hypodermic needle (fig. 4). A lancet is likely to make too large an opening or sever the blood vessel completely, resulting in excessive hemorrhage. For the test it is desirable that a small pool of blood be permitted to rise from the point of puncture (fig. 5), in order that the blood loop may be properly immersed for filling. It is good practice to disinfect the bleeding instrument before puncturing the wing vein. Care should be taken however, to wipe off the disinfectant with sterile cotton so that none of it may get into the blood that is to be tested.

TAKING THE BLOOD SAMPLE

Various instruments, including medicine droppers, celluloid paddles, etc., have been observed in use for taking up the blood for the test. All these are objectionable in that they do not measure correctly the quantity of blood to be used.

The ratio of blood to antigen should be within a definite range for the test mixture, consisting of 1 part of blood to 2 or 3 parts of antigen.

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With the standardized antigen dropper 0.05 cc. of antigen is delivered on the testing plate. This should be mixed with 0.02–0.025 cc. of blood. A loop for measuring the correct quantity of blood is usually supplied by the manufacturer of the antigen. A satisfactory loop may be made from a piece of nichrome wire, Brown & Sharp No. 20 gage, on one end of which may be made a loop, by wrapping it around a No. 20 steel machine drill, or a tenpenny wire nail. A convenient instrument consists of a handle of wood or metal, with the loop mounted on one end and a bleeding needle on the other (fig. 4).

With the loop the blood is taken up from the pierced wing vein. When submerged in the blood pool and then carefully withdrawn, the

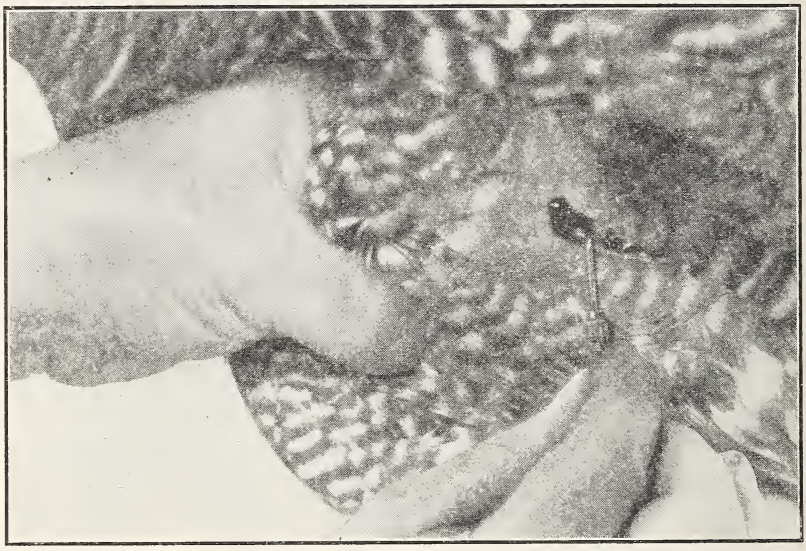

FIGURE 5.—The proper method of bleeding a chicken for the test. Note that the wing is being held with the left hand while puncture of the vein on under side of the wing is made with needle in right hand. The blood sample is taken from the small pool of blood shown at the point of puncture. The vein is exposed by plucking away several feathers.

loop comes away properly filled. On looking down edgewise at the filled loop the blood appears to bulge out. If insufficient blood has appeared on the surface, the skin should not be scraped with the loop in an effort to take up the blood, but another puncture should be made to bring sufficient blood to the surface. If the operator waits too long for a pool of blood to appear, clotting of the blood is likely to occur. This will be certain to interfere with a satisfactory test. In such case, the wing should be wiped clean with a cloth, and another puncture made to obtain a sufficient quantity of blood without delay. The loopful of blood is then stirred thoroughly into the drop of antigen, which has already been placed on the plate, and the mixture is spread out to a diameter of about 1 inch. The loop is then thoroughly rinsed in clean water, and dried on a clean cloth. It is not necessary to disinfect the loop.

MAKING THE TEST

Various types of testing equipment are in use. Ingenious testing boxes have been devised by a number of workers, some features of which are described and illustrated herein (figs. 6 and 7). For testing plates, the following materials have been used: Plain glass with a white paper background, plain glass with white paint on the under side, white opaque glass, and white porcelain. Any of these materials are satisfactory. However, the white opaque glass is the prevailing type of testing plate, and may be obtained in any size and thickness. A white glass plate approximately 7 by 9 inches and one-fourth inch thick is commonly used. This type of plate is convenient to handle and to cleanse and does not break easily. Plain glass without a white

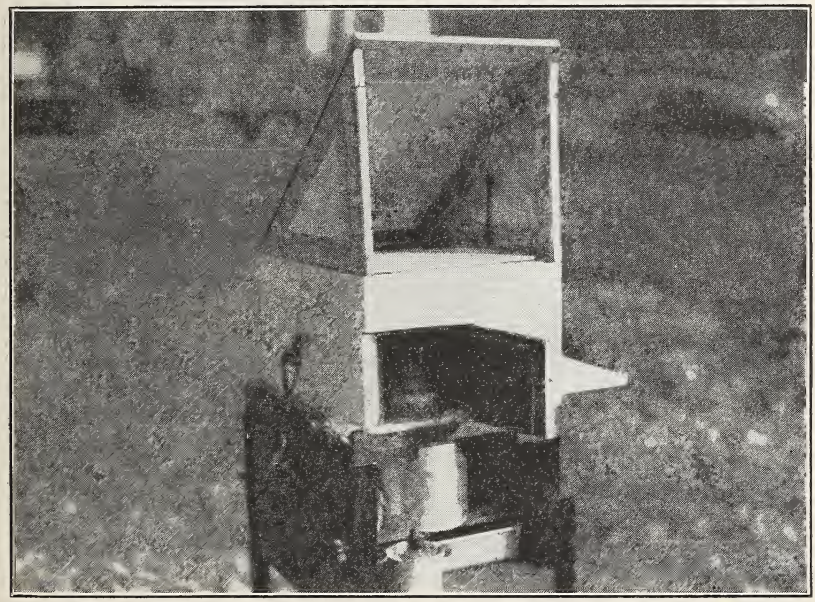

FIGURE 6.—A satisfactory testing box set up ready for operation. Note dust-and-wind shield on top of wooden frame covered with flexible glass substitute. Warm-water tank is concealed beneath white glass testing plate with alcohol lamp beneath to maintain warmth in cold weather. Compartments are provided also for can of water and soaking pan for used plates. The box is supported by slotted, adjustable angle-iron legs held in place with wing nuts at sides of box.

background, mirrors, and testing paper are not desirable. Paper is more or less porous and susceptible to absorption of moisture from the blood-antigen preparation and tends to wrinkle and pit, thus interfering with a satisfactory test. The alleged value of filing testing papers for future record has not been proved practicable as the true reactions cannot always be recognized after the preparation has dried. Plain glass without a white background gives poor visibility to the reaction. The mirror gives a double picture and throws a glare of reflected light into the tester's eyes.

Some commercial testing boxes provide plates that are ruled in squares, each square being intended as space for one test. The squares

must be large enough for a satisfactory test preparation. Unless the blood-antigen mixture can be spread over a diameter of an inch, approximately that of a 25-cent coin, the squares are too small to be satisfactory, and the reading is made difficult. It is obvious that if the blood and antigen are mixed and spread over an area about the size of a dime or a cent, the thickness of color thus piled up will prevent the tester from seeing the white background. Consequently a partial or border-line reactor may be entirely overlooked. In fact, the ruling of the plate is not at all necessary. It is better for the tester to acquire the habit of making correctly sized test preparations without the rulings. If the preparations are properly stirred and spread to a 1-inch

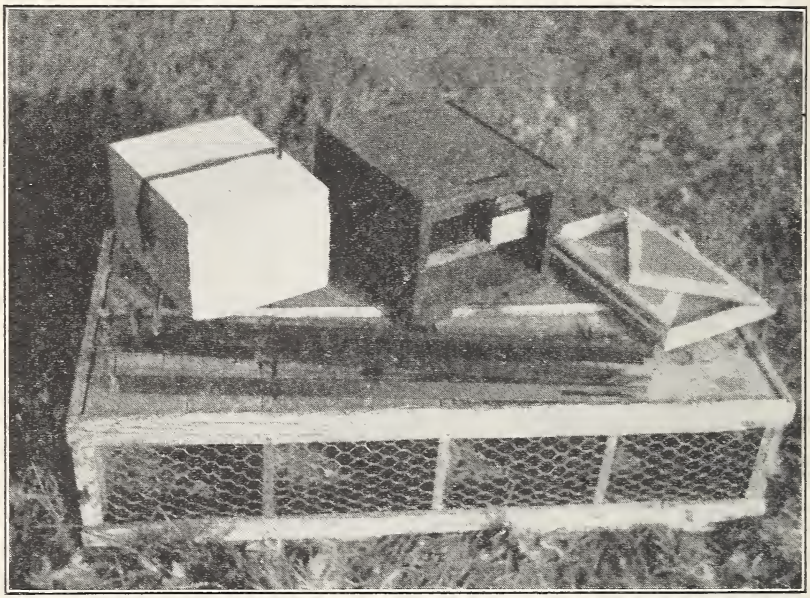

FIGURE 7.—Same equipment shown in figure 6 packed for transportation. The holding cage has eight compartments for birds that are being tested. Note folded dust-and-wind shield, also angle-iron legs detached from testing box.

circle, they will not tend to run together so readily when the plate is rotated in the process of mixing.

DROPPING THE ANTIGEN ON THE PLATE

The drop of antigen should be placed on the plate just prior to bleeding the bird. The antigen droppers supplied with the antigen by most of the manufacturers are calibrated to measure the correct quantity of antigen for the test, if held in a vertical position while dropping the antigen. The dropper should never be touched to the plate. Slanting positions of the dropper should be avoided as they tend to change the quantity of antigen delivered. If an insufficient drop is accidentally delivered, no attempt should be made to correct the quantity, but it should be wiped from the plate and another drop placed in the space. One drop of antigen at a time is the correct procedure. The common practice of placing six to eight drops of antigen on the plate at a time is not to be encouraged, because the

drops begin to evaporate immediately, and by the time the last few drops are utilized they have lost considerable moisture, and the test is no longer standard. Furthermore, a crust of dried antigen that does not mix readily with the blood may be formed around the edges of the last few drops. This crust may break up into clumps and it tends to produce a false reaction. It is advisable to have an excess of antigen drawn up into the dropper, and to press out any air column or bubbles in the tip of the dropper before delivering a drop of antigen on the test plate.

Upon adding the blood to the antigen on the plate, it is important that they be thoroughly and immediately mixed by stirring the loop into the antigen for 15 to 20 revolutions, in order to avoid the possibility of some clotting. In addition, the plate should be rotated a few times off and on during the course of the 2-minute interval, as other tests are added to it. It is more convenient to rotate the plate when it is free from the testing box. "Built in" plates necessitate rotating the testing box, a clumsy procedure. Many reactions will show up almost immediately when the blood and antigen are stirred together, others will be slower in appearing. So it is important that

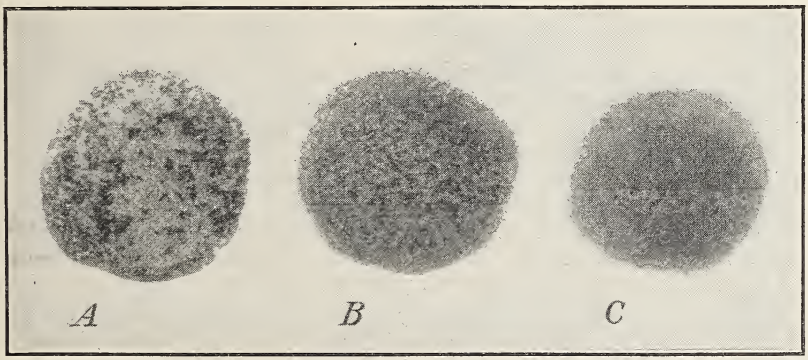

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FIGURE 8.—Reactions to the stained-antigen, rapid, whole-blood test: *A*, A positive reaction; *B*, a questionable or suspicious reaction; *C*, a negative reaction.

they be allowed a 2-minute interval. After 2 minutes the smear should be disregarded, since false reactions sometimes occur late.

READING THE REACTION

Various degrees of reaction are observed in this as in other agglutination tests (fig. 8). The greater the agglutinating power of the blood the more rapid the clumping and the larger the clump. A positive reaction consists in a clumping of the antigen in well-developed violet-colored flocculi surrounded by clear spaces. This reaction is easily distinguished against a white background. A somewhat weaker reaction consists of small but still clearly visible clumps of antigen surrounded by spaces only partially clear. These partial reactions should be regarded as suspicious, the same as similarly incomplete tube-method agglutination reactions, and the birds involved should therefore be eliminated from the breeding flock. Between this point and a negative reaction there sometimes occurs a very fine granulation barely

visible to the naked eye; this should be regarded as negative in making a diagnosis.

The very fine marginal flocculation which may occur just before drying up is also regarded as negative. In the case of a nonreactor the smear remains uniformly cloudy. The tester may have a number of successive test mixtures under observation without holding up the work to wait for results before proceeding to the next bird. As a result of experience with this antigen, it has been decided to regard as definitely positive only those reactions which appear within 1 minute after mixing the antigen and blood, while those which appear more slowly within 2 minutes are regarded as suspicious. In the practical application of this test, the suspicious birds are best removed along with the positive reactors.

If the birds are to be leg-banded, this is sometimes done after the reactors have been removed from the holding table and their tail feathers cut off. It is usually not advisable for the tester to band the birds, as this work should, if possible, be handled by an assistant as shown on cover page and in figures 2 and 3. The tester should not attempt to administer vaccines or remedies nor cull the flock while conducting the pullorum test.

KEEPING DUST OFF THE PLATE

Dust which settles on the plate may interfere with the test. Dust particles may take up stain from the antigen and may give the appearance of an agglutination reaction. It is therefore important, so far as possible, to control the dust factor. Tests that are conducted out of doors are usually not affected by dust. Birds should be prevented from flapping their wings. The testing table should be located to windward of the catching coop, if there is any appreciable atmospheric movement. A folding dust-and-wind shield (figs. 6 and 7) will be a help in controlling dust and evaporation.

If testing must be done in the poultry house, especially with litter on the floor, movements about the room and the excitement of the birds must be kept at a minimum. Birds released should be placed in another room or coop as they will begin to scratch in the litter as soon as released. If the room is very dry, the litter should be dampened with a garden sprinkler to lay the dust.

If, in spite of all these precautions, dust settles on the plate, the operator should at frequent intervals wipe the portion to be used with a piece of dry soft cloth. The plate should not be wiped with the bare hand, as this will tend to make the surface oily and unsuitable for a blood-antigen smear.

WARMING THE TESTING PLATE

The most favorable temperatures at which the test may be conducted are between 60° and 80° F. At temperatures warmer than this, rapid evaporation occurs. This should be avoided. In warm weather the test should be performed in a shaded place as sun heat may interfere with the test by causing rapid evaporation. Artificial heat should not be introduced into the test unless the atmospheric temperature is below 60°. Even in cold weather, only sufficient artificial heat should be used to raise the temperature of the test plate to the desirable range, between 60° and 80° F. Thermometers are not considered necessary, but if the plate feels warm to the under side of the wrist, it

is too warm for the test. It is preferable for the plate to feel slightly cool, but not cold.

Artificial heat may be provided in several ways. Some testing boxes are equipped with electric heating elements that may be connected to a storage battery or to house current. Others are equipped with flat warm-water cans of about 1 gallon capacity. Some of these are provided with removable canvas or pasteboard insulation to avoid excessive heat on the testing plate. Some have installed a small alcohol lamp beneath the warm-water can to maintain a moderate heat throughout the day (fig. 6). Chemical heat pads which become warm upon the addition of water are also used, but the heat from these pads is difficult to regulate.

CLEANSING THE PLATE

Provision must be made for cleansing the testing plate for further use. Nothing more than clean water need be used for this purpose. The application of soap, disinfectants, or cleansing compounds is undesirable as these may leave a residue which may affect subsequent tests. However, if the blood-antigen preparations roll up and refuse to spread properly, this may be an indication that there is a film of grease on the plate. In this case some soap powder or other suitable cleansing agent may be used, but the plate must afterwards be thoroughly rinsed and polished. Some testers keep a pail of water and a sponge or washcloth at hand, and either immerse used plates in the pail to soak before sponging them off; or they sponge the plate off clean for immediate re-use. The blood-antigen mixture is easily removed with clean, cool or tepid water. Hot water should not be used, as it may tend to coagulate the blood and render it difficult to remove from the plate. The plate should not be scraped with a metallic instrument nor cleansed with abrasives as this may scratch the surface and damage the plate.

After the plate has been cleansed, it should be polished dry with a clean cloth. A dish towel will do for this purpose provided it is clean. No lint or blood smears should remain on the plate. It is advisable, although not necessary, to have several plates so that, in case one is broken or proves difficult to cleanse, others will be available for continuation of the work. For ease of rotating as well as for facility of soaking and cleansing the plates, they should be easily removable from the testing box. Plates that are built into the box are difficult to cleanse, and also difficult to rotate.

DISPOSAL OF REACTORS

The National Poultry Improvement Plan requires that the pullorum-disease reactors "must be removed from the premises upon the completion of the test, and the premises must be immediately carefully cleaned and disinfected to the satisfaction of the official State agency." This clause is just as much a part of the pullorum control program as the blood-testing of the birds.

It is the usual practice for the tester to provide a reactor crate, into which all birds are to be placed that are to be removed from the flock. A good practice is to cut off the tail feathers of every reactor and remove any leg bands so that reactors may be distinguished and caught, at sight, in case of their escape. Upon conclusion

of the test, the reactor birds are immediately removed for disposal for slaughter purposes only. It is advisable for the pullorum testing agent to weigh the reactor birds in the presence of the flock owner. Instances have been known where reactor birds have been salvaged for egg-laying purposes. The official State agency may not always be able to assure itself that eggs so produced will not be offered for hatching purposes; therefore it is advisable to provide that reactors go to slaughter.

At the conclusion of the test at a poultry establishment, the equipment, such as catching crate and holding tables, should be cleaned and disinfected so as to avoid the possibility of disseminating any infection that may exist unsuspected in the flock. In this way flock testers will avoid criticism in case of outbreaks of disease and will also establish in the minds of their clients the proper respect for the pullorum control program. If the tester wears clean overalls and footwear that may be disinfected, he will further protect the interests of the flocks in which he is endeavoring to control pullorum disease.

CARE OF ANTIGEN

The stained antigen for the pullorum disease test contains a preservative that is intended to prevent decomposition or other change that may be caused by bacterial contamination. It is advisable, however, to use every possible precaution to prevent the contamination or spoiling of antigen. When not in use, the antigen bottle should be well stoppered and kept under refrigerator conditions, but antigen should not be allowed to freeze. During the testing of a flock the bottle should be kept stoppered except when necessary to place a drop on the plate. The bottle should be kept in the shade, as excessive heat or sunlight may damage the antigen. It is also advisable to have a block of wood with a hole in the center large enough to hold the antigen bottle, thus keeping it from upsetting, with danger of breakage or spilling of contents. Antigen should be used if possible from the original bottle. If it is necessary to pour from a stock container into a service bottle, care should be taken not to contaminate the antigen in any way. Antigen should be thoroughly shaken before being poured from one container into another and before the test is begun. The bottle should be restoppered and shaken about once every hour during the test, as there is a tendency for the killed bacteria in the antigen to settle to the bottom, leaving only the more fluid portions in the top of the bottle. The antigen should never be diluted or adulterated with any other material, as it is standardized during production and is intended to be used as it comes in the original package.

Antigen should not be kept from one season to another and should not be used after the expiration date indicated on the label. The antigen is a sterilized product and will not spread pullorum disease under any circumstances.

Commercial stained antigen for the rapid, whole-blood test for pullorum disease is produced and distributed by permission of the Secretary of Agriculture to whom was assigned the patent granted to J. M. Schaffer, an employee of the Department. Producers are required to adhere strictly to the Department's formula in making antigen and are also required to submit to the Bureau of Animal

Industry a preliminary sample of every lot made. These samples are subjected to a critical examination and, if satisfactory, are included in a list of antigens approved for the control of pullorum disease under the National Poultry Improvement Plan. These lists are supplied periodically to the official State agencies responsible for pullorum-disease control in participating States.

PROBLEMS ENCOUNTERED IN PULLORUM TESTING

When properly applied, the agglutination test is one of the most reliable known to veterinary science; but defective technique, unskilled interpretation, or the use of unsuitable equipment or bad antigen are likely to lead to erroneous results.

Occasionally an alarmingly high percentage of pullorum reactors will occur unexpectedly in a previously tested flock. One or more of the following factors may be responsible:

The flock may not have had the proper sanitary surroundings. Premise infection or reactor birds left on the premises from previous tests may have caused the development of new reactors.

The flock may have been recently vaccinated with a vaccine containing *Salmonella gallinarum*, the fowl typhoid organism. The blood of fowls so vaccinated may cross-agglutinate pullorum-disease antigen for some time afterwards. Flocks recently vaccinated for fowl typhoid should not be tested for pullorum disease for at least 60 days thereafter.

Defective antigen or technique employed on previous tests may have failed to remove pullorum disease from the flock.

The antigen may have gone bad or may be too sensitive. It is advisable to check a drop of antigen without blood, on the plate, for spontaneous or false agglutination, before proceeding with the test.

The tester may be reading the tests too closely and erroneously interpreting the late powdery formations or marginal flocculations as pullorum reactions.

Excessive evaporation may be causing premature marginal flocculations.

The tests may be read too late. Readings should not be made after 2 minutes.

Antigen may for some reason lose its sensitivity and, although such cases are rare, it may fail to detect pullorum disease. If there is any doubt of the potency of an antigen, it is well to check it on a few known reactor birds before proceeding with the test.

Whenever there is any question concerning the potency or specificity of a supply of antigen, it is advisable for the tester to communicate with his official State agency regarding having the antigen checked, or else set it aside and obtain a new supply of approved antigen.

Testers should not attempt to make a record for speed when testing a flock. The number of birds tested daily is less important than the thoroughness and care with which the test is done.

Sanitation in the poultry house and hatchery is an important part of pullorum disease control. In spite of all precautions the infection will sometimes find its way into the hatchery. The disinfection of equipment will go far to prevent its spread.

DISINFECTION OF INCUBATORS AND BROODERS

The down of chicks which hatch from pullorum-disease-infected eggs is contaminated with pullorum organisms. When the down dries and is shed by the chick, the infection is spread in the incubator, or hatcher, when the latter equipment is used. The excreta of such chicks are also infectious. Other infections, such as those of bronchitis, coryza, omphalitis (mushy-chick disease), and lymphomatosis, sometimes gain entrance and become serious problems to the hatcheryman. Losses from these causes may to some extent be controlled by sanitation. Dirty hatching eggs or eggs from diseased breeding flocks should not be introduced into the incubator.

Formaldehyde fumigation of the incubator, hatcher, or brooder is of material assistance in the control of such infections. However, before undertaking to fumigate or otherwise disinfect, the equipment or room to be so treated should first be thoroughly cleansed. At the beginning of the hatching season, as well as between hatches, the machines, rooms, and equipment should be cleansed and disinfected before any eggs are brought in. Egg trays, nursery trays, and other readily removable parts should be taken out and scrubbed with a 2-percent solution of commercial lye. Chick down, droppings, and shell fragments should be removed from the incubator with a brush or vacuum cleaner (fig. 9). Dead chicks and unhatched eggs should be burned after each hatch. The initial fumigation, as well as later fumigations between hatches, should be done with double the quantities of chemicals recommended for routine use. Double-strength fumigation is too strong to use while chicks are present, but will not injure hatching eggs.

A routine procedure for fumigating incubators has been carefully worked out by Robert Graham of the University of Illinois, and other investigators. For each 100 cubic feet of space to be fumigated, 35 cubic centimeters (about 1⅛ fluid ounces) of formalin is mixed with 17.5 grams (about ⅝ ounce) of potassium permanganate to liberate the gas. Because the action of these two substances when mixed is rapid and violent, and because formaldehyde gas in strong concentration is very injurious and dangerous to human beings and other mammals, careful and thorough preparations must be made to insure safe and successful fumigation.

It is advisable to purchase formalin (formaldehyde solution) only in quantities that will be utilized soon, as some danger is involved in keeping this poisonous material around. If it must be stored, precautions should be taken to keep the container tightly corked and protected from upsetting or breakage, and that children, pet animals, and irresponsible persons do not have access to it. Persons using this chemical should wear rubber gloves, as formalin is extremely destructive to the skin or body tissues. Care should be taken to prevent formalin from spattering into the eyes while the drug is being poured out for use. During all of the operations involved in fumigation, the wearing of a suitable gas mask would also be an advisable precaution.

The potassium permanganate crystals are put in a relatively large pan which is placed in a high-walled pan, tub, or pail set on the floor of the incubator as close as possible to the intake pipe. The damper is left wide open so that the gas can penetrate to all parts of

the incubator. The purpose of the outer container is to catch any hot residue that may be expelled from the inner container during the reaction.

Preparations must be made in advance to have the humidity in the machine up to at least 68 percent as indicated by a wet bulb reading of 90° F. when the dry bulb reads 100°. High temperature is another

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FIGURE 9.—Removing chick down, droppings, and shell fragments from the incubator by the aid of a vacuum cleaner, preparatory to disinfection of the incubator.

important factor in effective formaldehyde fumigation. Incubating temperature is satisfactory.

FUMIGATING THE HATCHED CHICKS

If fumigation is done while chicks are in the incubator, precautions should be taken to place the chemicals where the gas will be thoroughly mixed with the air before reaching the chicks. Chicks 48 hours old or older or chicks that have dried off in the incubator should not be exposed to fumigation with formaldehyde gas, as they may be injured by such exposure.

When every preparation has been completed, the required quantity of formalin is quickly poured over the potassium permanganate crystals and the door of the incubator closed immediately. The operator

must be prepared to leave the room promptly and close the room door tightly, as the fumes that escape are dangerous to human beings. However, just before leaving the room, the operator should turn on the incubator fans in order to distribute the gas throughout the closed incubator.

The doors of the incubator should be opened at the expiration of 3 hours and the chicks removed to clean quarters. The irritating effect of the formaldehyde gas may be neutralized at that time by spraying or sprinkling the interior of the incubator (but not the chicks) with concentrated ammonia water, U. S. P. (not household ammonia), using one-half as much of the ammonia as the formalin used. This procedure is carried out only for the comfort of the workers.

Three fumigations, 12 hours apart, should be made during the course of the hatch. Any chicks that have dried off should be removed from the incubator before each fumigation. The first fumigation is done shortly after the first chicks have come out of the eggs. Mortality in fumigated chicks is said to be confined largely to weaklings and pullorum-infected birds.

FUMIGATION WITH FORMALIN ALONE

Dr. Graham also recommends the following method of fumigation as simpler, more economical, and equally as effective as the formalin-permanganate of potassium method:

Use 20 cubic centimeters of formalin for each 100 cubic feet of space to be fumigated. Avoid using old formalin that has been left uncorked and that may have deteriorated. The use of a gas mask and rubber gloves during the operations of fumigation is recommended.

Cut cheesecloth into yard squares and dip in the formalin until all has been absorbed. Then hang the cheesecloth near the fan or fans but not where it will interfere with their movement. Close the incubator and turn on the fans. The same precautions are recommended when using this method as when using the combination of formalin and potassium permanganate.

Let the formalin-soaked cheesecloth remain in the incubator at least 3 hours, then remove all dry chicks.

Give one fumigation when one-tenth to one-fifth of the chicks are out of the eggs, and the second 12 to 15 hours later. Two fumigations are sufficient by this method.

Humidity and temperature requirements are the same as for the formalin-potassium permanganate method previously described.

Empty incubators, incubator rooms, or other equipment may be fumigated by wetting or spraying thoroughly the exposed surfaces with a 5-percent solution of formalin and then leaving the door closed for 12 hours at a temperature not below 60° F. Chick brooders and brooder rooms, when not in use, may be disinfected in a similar manner. Additional information on the fumigation of an incubator may be obtained from the manufacturer.

DISINFECTION OF POULTRY YARDS AND BUILDINGS[2]

The soil may be disinfected by wetting it thoroughly with a solution made by dissolving 1 pound of commercial lye and 2½ pounds of

[2] Additional information applicable to the disinfection of poultry premises is contained in Farmers' Bulletin 926, Some Common Disinfectants, and Farmers' Bulletin 954, The Disinfection of Stables, which may be obtained from the Superintendent of Documents, Government Printing Office, Washington, D. C., at 5 cents each.

water-slaked lime in 5½ gallons of water, using from ½ to 1 gallon of the solution per square yard of soil surface, depending upon the absorbent quality of the ground. It is essential, however, that all refuse matter be removed from the surface and burned or buried before undertaking to disinfect poultry runs. This same solution is applicable to the disinfection of poultry houses and equipment. If the solution is not used at once, it should be tightly covered to prevent deterioration. This solution is cheap, odorless, and destructive to many kinds of disease germs. On prolonged contact, however, it may be injurious to painted or varnished surfaces, and to some fabrics. It is corrosive to aluminum, but relatively harmless to the metallic fixtures ordinarily found about chicken houses, and to wooden construction or equipment. Precautions should be taken by the operator not to get the solution on the skin or in the eyes.

LIST OF EQUIPMENT FOR THE STAINED-ANTIGEN, RAPID, WHOLE-BLOOD TEST

White, hard-surface testing plate.
Flat can for warm water, or other warming device.
Sun-and-wind shield for test plate.
Antigen with standard dropper to deliver approximately 0.05 cc. of antigen.
Stylet or bleeding instrument.
Small jar of 95-percent alcohol for disinfecting stylet.
Blood loop standardized to deliver approximately 0.025 cc. of blood.
Small jar of water for rinsing loop.
Several pieces of dry, clean absorbent cloth for drying and polishing testing plate and for drying blood loop.
Clean washcloth or sponge.
Pail of clean water for washing plate.
Holding table for retaining birds while under test.
Leg bands for nonreacting birds.
Pair of banding pliers.
Scissors or tin snips, for cutting tail feathers and leg bands from reactors.
Scales for weighing reactors.
Retaining coops for reactors.
Catching coop for holding birds until ready for testing.

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